LXI. OBSERVATIONS ON THE CARBOHYDRATE METABOLISM OF TUMOURS.

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The studies of Warburg and his collaborators on the carbohydrate metabolism of surviving tumour tissues [1926] have dealt mainly with two transplantable strains of rat tumours, Jensen's rat sarcoma and Flexner's rat carcinoma, and the Rous chicken sarcoma.

On the basis of metabolism values found for a large number of these tumours, together with those obtained for a more limited series of human neoplasms, Warburg has suggested several generalisations, showing characteristic relationships between the magnitudes of the respiration and the aerobic and anaerobic glycolysis. The constant result which emerged was the abnormally high value of the anaerobic glycolysis as compared with the respiration.

Assuming that the oxygen utilised was functioning at its maximum efficiency in causing the removal or non-formation of lactic acid under aerobic conditions, the respiration was found inadequate to check the glycolysis completely, a relatively large excess fermentation remaining.

Representing this excess fermentation by U, the respiration by Q_{O_2}, and the anaerobic glycolysis by $Q_M^{N_2}$

$$U = Q_M^{N_2} - 2\,(Q_{O_2}).$$

Positive values for U were invariably found for the tumours quoted.

Aerobic glycolysis is not a specific feature of tumour tissue. Warburg [1929] has recently summarised examples of normal tissues possessing aerobic glycolysis, and Crabtree [1928] showed it to be a property of certain pathological overgrowths associated with intracellular viruses.

During the last year, as material has become available, the carbohydrate metabolism of several strains of transplantable mouse tumours, propagated in this laboratory, has been measured, and the results are collected in Table I. Although Warburg no longer adheres to the classification of tissues on the basis of a positive or negative value of U, throughout this communication the value will be introduced as affording an arbitrary standard of comparison, placing the present measurements in line with those previously recorded.

The manometric technique elaborated by Warburg has been followed throughout.

Table I. *Carbohydrate metabolism of mouse tumours.*

Tumour	Q_{O_2}	$Q_M^{O_2}$	$Q_M^{N_2}$	Meyerhof quotient	U
Crocker sarcoma	−10·6	+14·8	+30·4	1·5	+ 9·2
,,	−14·6	+15·6	+25·2	0·6	− 4·0
,,	−30·9	+20·7	+31·7	0·4	−30·1
,,	−13·9	+15·0	+26·5	0·8	− 1·3
,,	− 8·2	+16·3	+26·5	1·2	+10·1
,,	−16·5	+16·2	+30·5	0·9	− 2·5
,,	− 7·6	+13·2	+30·6	2·3	+15·4
,,	−15·5	+17·3	+24·8	0·5	− 6·2
,,	−24·4	+19·4	+24·9	0·2	−23·9
,,	−11·9	+16·7	+22·1	0·5	− 1·7
,,	− 6·5	+12·6	+23·2	1·6	+10·2
,,	−20·7	+19·7	+31·3	0·6	−10·1
,,	−21·4	+20·9	+33·3	0·6	− 9·5
Tar carcinoma (Sp) 2146	−19·3	+19·6	+23·5	0·2	−15·1
,,	−21·3	+18·4	+24·5	0·3	−18·1
,,	−15·2	+14·3	+24·7	0·7	− 5·7
,,	−31·0	+18·6	+25·7	0·2	−36·3
,,	−19·5	+11·6	+24·0	0·5	−15·0
,,	−13·3	+ 8·5	+24·0	0·9	− 2·6
37 sarcoma	−10·9	+12·6	+23·5	1·0	+ 1·7
,,	− 9·8	+15·3	+29·4	1·5	+ 9·8
,,	−12·2	+ 6·5	+26·6	1·6	+ 2·2
,,	−12·1	+ 6·9	+27·6	1·6	+ 3·4
,,	−22·3	+14·4	+32·4	0·8	−12·2
,,	−24·3	+14·8	+33·4	0·7	−15·2
Tar sarcoma Bonné	−15·9	+ 9·1	+20·7	0·7	−11·1
,,	−13·9	+ 8·6	+20·8	1·0	− 7·0
,,	− 9·9	+12·3	+24·9	1·3	+ 5·1
Tar carcinoma 173	−12·0	+15·2	+30·7	1·3	+ 6·7
,,	−13·0	+16·3	+24·1	0·6	− 1·9
,,	−20·1	+15·4	+31·9	0·8	− 8·3
,,	−16·6	+20·7	+28·9	0·5	− 4·3
,,	−17·0	+19·5	+28·9	0·5	− 5·1
,,	−16·7	+17·0	+36·0	1·1	+ 2·6
,,	−14·4	+18·9	+37·0	1·2	+ 8·2
,,	−14·5	+10·2	+21·5	0·8	− 7·5
,,	− 8·2	+ 6·7	+21·5	1·8	+ 5·1
,,	−10·0	+ 8·2	+26·5	1·8	+ 6·5
,,	−18·3	+11·6	+27·5	0·8	− 9·1
Sarcoma 2529	−14·8	+14·9	+32·6	1·2	+ 3·0
,,	− 5·6	+12·8	+30·6	3·5	+19·4
,,	−16·5	+17·8	+36·3	1·1	+ 3·3
,,	−19·6	+22·1	+36·9	0·8	− 2·3
,,	−16·0	+13·5	+28·3	0·9	− 3·7
,,	−11·6	+12·7	+28·3	1·3	+ 5·1
,,	−10·9	+14·8	+29·7	1·4	+ 7·9
,,	−12·9	+15·1	+28·7	1·1	+ 2·9
Glycogen-carcinoma 113	−15·5	+ 6·8	+12·6	0·4	−18·4
,,	−12·5	+ 5·0	+12·7	0·6	−12·3
,,	− 8·3	+ 3·4	+14·4	1·3	− 2·2
,,	− 7·6	+ 2·8	+14·1	1·5	− 1·1
,,	−11·4	+ 4·3	+16·1	1·0	− 6·7
Melanotic sarcoma	− 5·6	+ 7·2	+23·7	2·9	+12·5
,,	− 8·8	+ 4·3	+15·3	1·3	− 2·3
,,	− 7·7	+ 4·4	+15·1	1·4	− 0·3
,,	−11·7	+ 7·0	+ 8·9	0·2	−14·5

The composition of the saline medium used, unless otherwise stated, was:

Salt	Moles per litre
NaCl	0·121
KCl	0·0025
$CaCl_2$	0·0018
$NaHCO_3$	0·025

Glucose was added in a concentration of 0·2 %, and the appropriate gas phase was 5 % CO_2 in O_2 or in N_2.

The method of expressing results is that used by Warburg and is explained in an earlier communication of the present author [1928].

Though every precaution was taken to ensure efficient metabolism, by using thin sections which permit perfect diffusion of metabolites, and by removal of all traces of necrosis visible to the naked eye, the results obtained were widely divergent in character.

The great variability in the absolute and relative magnitude of the metabolism values is not merely between the various strains investigated, but is apparent among different tumours of the same strain.

About one-third show values comparable to those found for Jensen's rat sarcoma and Flexner's rat carcinoma (see Table VI). The majority deviate from these standards, chiefly by exhibiting a higher respiration, both in its absolute value and also in its relation to the aerobic and anaerobic glycolysis. The relationship is emphasised by the negative value of U in numerous cases.

Many factors could conceivably operate in contributing to these abnormal findings: the generally more active metabolism of the mouse compared with larger animals, the variations in the environment at the site of growth, the possible fluctuations in the respiratory quotient, or the effectiveness of the blood supply. Also, in some tumour strains phases of rapid growth alternate with phases of depression with slow growth, and the position of the tumour in such a cycle at the time of the experiment would probably exert its influence.

Some of these possibilities were tested, and the results are recorded in later sections of this communication. Warburg postulates a disturbance of respiration as being the fundamental cause of the development of aerobic glycolysis. In many of the tumours included in the above table the respiration is very high, exceeding that of any tissue, normal or malignant, so far examined. In such cases, apparently, the Pasteur reaction fails to function, and until more is known of the mechanism by which respiration checks fermentation, the value of hypotheses involving conceptions of different kinds of respiration is doubtful.

The measurement of the respiratory quotient.

The Ringer solution used for the simultaneous measurement of respiration and aerobic glycolysis closely approximates, in its salt content and bicarbonate concentration, to physiological standards. The p_H is regulated by using 5 % CO_2 in O_2 as the gas phase, and for saturation of the media. The Henderson-Hasselbalch equation [Hasselbalch, 1917] then shows the p_H to be 7·35 at 37·5°. The procedure adopted for the measurement of the respiratory quotient involving absorption of CO_2 by potash is obviously inapplicable if these more accurate physiological conditions are to be maintained.

In the method utilised below, determinations of the respiratory quotient were made under the more favourable conditions used for simultaneous estimations of respiration and aerobic glycolysis.

Since Negelein [1925] has shown that the glycolysis effected by tumour tissue is a pure lactic acid fermentation and can be accurately measured by the fall in the bicarbonate concentration of the Ringer solution used experimentally, the respiratory CO_2 is obtained by deduction of the CO_2 evolved due to glycolysis (found by estimation of the bicarbonate concentration at the end of the metabolism experiment), from the total CO_2 evolved due to glycolysis and respiration and measured manometrically.

The following protocol will illustrate the method. In addition to the three manometers normally used for determination of aerobic metabolism, an extra one (Z) is required to measure the lowering of bicarbonate concentration during the 15 minutes' preliminary shaking until equilibrium is attained, *i.e.* to the time $t°$. This estimation, after correction for differences in weights of tissue used, serves as a control for the vessel Y in which the bicarbonate concentration is measured after 60 minutes' metabolism from the time $t°$. By rapid manipulation, the section of tissue can be removed from the Ringer solution in about 20 seconds, causing an error of no more than 0·5 %.

Protocol I.

Mouse tar carcinoma 173. Temp. = 37·5°. Gas phase = 5 % CO_2 in O_2.

	Vessel O	Vessel X	Vessel Y	Vessel Z
	Ringer thermo-barometric control $V_F = 3$ cc.	$V_F = 3{\cdot}5$ cc. $V_G = 4{\cdot}08$ cc. $K_{O_2} = 0{\cdot}365$ $K_{CO_2} = 0{\cdot}554$	$V_F = 1{\cdot}0$ cc. $V_G = 6{\cdot}28$ cc. $k_{O_2} = 0{\cdot}554$ $k_{CO_2} = 0{\cdot}608$	For bicarbonate estimation at $t°$ $V_F = 1{\cdot}0$ cc. $V_G = 5{\cdot}85$ cc.
Time	Dry wt. of tissues	3·85 mg.	3·73 mg.	5·18 mg.
$t°$	Shake 15′ to attain equilibrium	—	—	Section removed
$t° + 60'$	Shake for 60′	Increase of pressure $H = +43$ mm.	Increase of pressure $h = +57$ mm. Section removed	—
	Manometric estimation of bicarbonate concentrations	—	$B_2 = 487$ mm.³	$B_1 = 518$ mm.³

From the increases of pressure observed in the manometers attached to the vessels X and Y, the gas exchange is calculated in the usual way.

x_{O_2} = mm.³ O_2 consumed in vessel Y in 60′ = 27·6 mm.³

$x^{O_2}_{CO_2}$ = mm.³ CO_2 evolved (from glycolysis and respiration) in vessel Y in 60′ = 64·6 mm.³

The bicarbonate estimations are carried out with aliquot portions of the Ringer solutions from vessels Y and Z, together with a concurrent estimation of the bicarbonate concentration of the standard Ringer solution. The manometric method used is described by Warburg [1926]. As a check on the value obtained for vessel Y, the bicarbonate concentration in vessel X may similarly be determined. This was not carried out as a routine procedure since several such preliminary estimations for checking purposes showed it to be superfluous.

Expressing the bicarbonate concentrations as mm.^{3} CO_2 per cc. solution,

$B_0 = C_{NaHCO_3}$ of standard Ringer solution = 558 mm.^{3}

$B_1 = C_{NaHCO_3}$ at $t°$ = 518 mm.^{3} (vessel Z).

$B_2 = C_{NaHCO_3}$ at $t° + 60'$ = 487 mm.^{3} (vessel Y).

Therefore

$$\text{respiratory quotient} = \frac{x^{O_2}_{CO_2} - \left[B_0 - \frac{3{\cdot}73}{5{\cdot}18}(B_0 - B_1) - B_2\right]}{x_{O_2}}$$

$$= 0{\cdot}812.$$

Table II gives a series of the results obtained, with the simultaneously determined metabolism quotients.

Table II. *Simultaneous measurements of the carbohydrate metabolism and the respiratory quotient.*

Tumour	A Respiration Q_{O_2}	B Aerobic glycolysis assuming R.Q. is unity $Q_M^{O_2}$ (1)	C Aerobic glycolysis based on R.Q. found $Q_M^{O_2}$ (2)	D Anaerobic glycolysis $Q_M^{N_2}$	E Respiratory quotient
Tar carcinoma 173	−12·3	+10·8	+14·1	+33·4	0·735
,,	−10·2	+12·0	+14·2	+23·4	0·790
,,	− 7·4	+ 9·9	+11·3	+15·3	0·812
Tar carcinoma 2146	−19·2	+13·9	+ 9·7	+26·3	1·220
,,	−14·8	+11·7	+12·8	+20·6	0·772
,,	−15·2	+13·5	+14·7	+27·4	0·923
Crocker sarcoma	−11·7	+10·2	+12·1	+24·0	0·839
,,	− 8·8	+ 6·8	+ 8·5	+16·3	0·805
,,	−13·2	+10·6	+15·2	+22·8	0·649
Jensen's rat sarcoma	− 5·9	+ 9·4	+11·0	+21·6	0·722
,,	−12·2	+11·1	+13·6	+21·3	0·794
,,	−12·8	+14·5	+19·1	+26·5	0·638

The aerobic glycolysis has hitherto been derived by deducting the measured respiration from the measured total CO_2 evolved through the operation of the combined respiratory and glycolytic processes. The assumption has been made by Warburg and other workers with these methods that the respiratory quotient is unity, under the conditions of the manometric experiment. In column B the aerobic glycolysis is given, as reckoned on this basis.

In column C the aerobic glycolysis is calculated from the value of the respiratory quotient experimentally obtained. Since the respiratory quotients, with one exception, are less than unity, the aerobic glycolysis thus found is higher than that calculated on Warburg's assumption. The effectiveness of the respiration in checking glycolysis is consequently less pronounced than is indicated by the Meyerhof quotients in Table I.

Effect of glycolysis on respiration.

In connection with an investigation into the possibility of tumour tissue metabolising pentoses, results were obtained which suggest that the glycolytic activity of tumours may act as a partial check on their respiratory powers.

The metabolism values included in Tables III and IV show that tumour tissue does not utilise xylose. The magnitudes of the respiration, whether measured in media free from sugar or with xylose added, are almost identical. No splitting of xylose occurs, with formation of acidic products, either aerobically or anaerobically.

(*a*) *Respiration measurements.* These were carried out in Ringer solution containing 0·0025 mol. of sodium bicarbonate per litre. The initial p_H of the solution was approximately 8·2. Concentrated potash was used as absorbent for CO_2. Two series of tissues were used in these measurements; eleven transplantable animal tumours, and rat liver and kidney, representative of normal tissues.

Table III *a* summarises the findings with tumour tissues.

Table III *a*. *Respiration of tumour tissues in Ringer solution, with and without sugar additions.*

C_{NaHCO_3} = 0·0025 mol. per litre. Gas phase O_2.

Tissue	Q_{O_2} Glucose added 0·2 %	Q_{O_2} Xylose added 0·2 %	Q_{O_2} No sugar added
Tar carcinoma 2146	−10·7	−12·1	−12·6
,,	−13·1	−16·6	−16·9
Jensen's rat sarcoma	−14·3	−16·6	−16·9
,,	−11·1	−13·9	−12·5
,,	− 9·8	−11·0	−11·1
,,	−10·4	−13·3	−13·1
,,	−12·1	−14·0	−14·3
,,	− 7·2	− 9·0	− 9·2
,,	−14·1	−16·7	−16·9
,,	−10·8	−13·1	−12·7
,,	− 9·7	−11·0	−11·2
Average	−11·2	−13·4	−13·4

With glucose-containing Ringer solution, the values obtained were consistently somewhat lower than those obtained in the absence of glucose.

The average lowering in the examples recorded was about 10 %.

Table III *b* shows the results obtained with normal tissues. Though the measurements were made under identical conditions, the effect of the addition of glucose was not to lower the respiration but, on the average, to raise it slightly.

Table III *b*. *Respiration of normal tissues, with and without sugar additions.*

C_{NaHCO_3} = 0·0025 mol. per litre. Gas phase O_2.

Tissue	Q_{O_2} Glucose added 0·2 %	Q_{O_2} Xylose added 0·2 %	Q_{O_2} No sugar added
Mouse liver	− 8·8	− 8·2	− 6·6
Rat liver	−10·5	− 9·8	−11·1
,,	− 7·3	− 7·6	− 6·4
,,	−11·7	−12·0	−12·1
,,	− 9·2	− 8·9	− 8·9
,,	− 6·1	− 5·2	− 5·5
Rat kidney	−21·6	−20·3	−20·0
,,	−16·6	−15·0	−15·0
,,	−16·6	−17·6	−14·9
,,	−22·6	−22·2	−21·4
Average	−13·1	−12·7	−12·2

Since the arbitrary conditions under which these measurements of respiration were made deviate considerably from normal physiological conditions, particularly with respect to the p_H and the bicarbonate concentration, their general significance is doubtful. The implied checking of respiration by glycolytic activity was tested under more appropriate conditions, which are recorded in the following section.

(*b*) *General metabolism measurements.* The standard solutions for the simultaneous determination of respiration and aerobic glycolysis were employed, with $C_{NaHCO_3} = 0{\cdot}025$ mol. per litre and $p_H = 7{\cdot}35$.

Table IV gives a summary of the results obtained.

Table IV. *Comparison of tumour metabolism in presence of glucose and xylose.*

$C_{NaHCO_3} = 0{\cdot}025$ mol. per litre. Gas phase = 5 % CO_2 in O_2.

	Glucose added 0·2 %		Xylose added 0·2 %		
Tissue	Q_{O_2}	$Q_M^{O_2}$	Q_{O_2}	$Q_M^{O_2}$	$Q_M^{N_2}$
Crocker sarcoma	− 8·3	+12·1	−10·8	−	+1·2
,,	−10·6	+14·5	−11·4	−	+1·1
,,	−13·6	+15·5	−14·5	−	+1·6
,,	− 9·9	+16·8	−12·1	−	+0·8
,,	−15·8	+17·8	−15·8	−	+1·0
Jensen's rat sarcoma	−11·0	+ 9·3	−13·1	−	+1·3
,,	−11·6	+12·1	−13·7	−	+0·9
,,	− 9·8	+ 8·9	−11·2	−	+1·2
Average	−11·3	+13·4	−12·8	−	+1·1

Tissues from the same animal were used in concurrent estimations in the presence of glucose and of xylose. The results show an appreciable lowering of the respiration, about 12 % on the average, in the glucose-containing Ringer solution when compared with the xylose-containing Ringer solution. With xylose present no acid formation results through sugar splitting, either aerobically or anaerobically.

The tentative conclusion is that glycolytic activity exerts a significant checking effect on the capacity for respiration of tumour tissue.

Influence of environment on the carbohydrate metabolism of Jensen's rat sarcoma.

A few observations of the carbohydrate metabolism of Jensen's rat sarcoma were made with the intention of confirming the numerous consistent values found by Warburg and his collaborators.

Anomalous results were obtained, a tendency for the respiration to be high with respect to the anaerobic glycolysis being often noticed. The value of U was often negative. In Table V are collected the results of a few of these preliminary determinations.

The tumour strains of the Imperial Cancer Research Fund are propagated by subcutaneous grafts in the flank.

Table V. *Jensen rat sarcoma.*

Q_{O_2}	$Q_M^{O_2}$	$Q_M^{N_2}$	Meyerhof quotient	U
−15·2	+13·5	+26·2	0·8	−4·2
−11·7	+10·2	+24·0	1·2	+0·6
−13·2	+10·6	+20·1	0·7	−6·3
−12·2	+11·1	+18·8	0·6	−5·6
−12·8	+14·5	+26·5	1·0	+0·9
−10·9	+ 9·3	+21·6	1·1	−0·2

The work of Campbell [1926] on tissue oxygen and carbon dioxide tensions has shown, by the method of gas injection, that the tensions of these gases vary in different parts of the body. Campbell and Cramer [1928] showed that rapidly growing implanted tumours in rats and mice show a greatly diminished rate of growth during prolonged exposure to low oxygen pressures. These observations suggested the possibility that the abnormal results recorded in Table V might be due to effects determined by the site of transplantation. Accordingly comparative series of metabolism measurements were made on two series of tumours, one series transplanted subcutaneously and the other intraperitoneally. The tumour used was Jensen's rat sarcoma, the rats chosen being of about the same age, 2 to 3 months. The inoculations were made with material from the same tumour and at the same date.

The tumours grown intraperitoneally are relatively more haemorrhagic than those grown subcutaneously and develop as a number of nodules adherent to neighbouring surfaces. As growth proceeds, these nodules unite, forming a large coherent mass. Isolated nodules provide the best material for metabolism experiments; microscopically they show numerous mitotic figures and no necrotic areas are visible.

The results are collected in Table VI.

Table VI. *Comparison of metabolism of Jensen rat sarcoma when transplanted subcutaneously and intraperitoneally.*

	Subcutaneous transplantations				Intraperitoneal transplantations			
	Q_{O_2}	$Q_M^{O_2}$	$Q_M^{N_2}$	U	Q_{O_2}	$Q_M^{O_2}$	$Q_M^{N_2}$	U
	−19·3	+12·3	+28·4	−10·2	− 6·2	+12·6	+31·6	+19·2
	−16·1	+19·6	+32·6	+ 0·4	− 7·6	+11·9	+29·0	+13·8
	−16·4	+16·8	+32·8	0	−12·3	+15·1	+26·9	+ 2·3
	−13·6	+13·7	+27·6	+ 0·4	−11·1	+15·4	+34·0	+11·8
	−18·9	+22·5	+31·6	− 6·2	− 8·6	+14·2	+25·4	+ 8·2
	−19·3	+20·1	+33·4	− 5·2	−11·1	+15·0	+25·4	+ 3·2
	−15·2	+14·1	+27·3	− 3·1	−13·3	+22·3	+37·5	+10·9
	−16·6	+15·5	+27·3	− 5·9	−10·3	+22·2	+37·5	+16·9
	−19·1	+25·8	+36·3	− 1·9	−12·3	+ 8·6	+25·0	+ 0·4
	−27·9	+26·5	+36·4	−19·4	− 9·6	+ 8·4	+24·7	+ 5·5
	−18·5	+24·5	+34·8	− 2·2	−12·4	+17·9	+33·6	+ 8·8
	−14·2	+24·2	+34·9	+ 6·5	−12·9	+17·1	+32·8	+ 7·0
	−15·3	+18·0	+30·6	0	−15·7	+17·2	+34·9	+ 3·5
	−12·0	+17·5	+34·5	+10·5	−15·9	+17·2	+34·9	+ 3·1
	−16·9	+23·3	+34·9	+ 1·1	−12·6	+17·4	+37·3	+12·1
	−16·7	+25·4	+30·9	− 2·5	− 7·0	+18·4	+35·3	+21·3
	−18·1	+25·2	+33·5	− 2·7	—	—	—	—
Av.	−17·3	+20·3	+32·2	− 2·4	−11·2	+15·7	+31·6	+ 9·2

A consideration of these results leads to the following conclusions.

1. All the tumours grafted intraperitoneally show a carbohydrate metabolism conforming to that found by Warburg. A positive U, or excess fermentation, is a common property.

2. A large majority of the tumours grafted subcutaneously show a value for U which is either negative or near zero.

3. The magnitude of the respiration of the tumours grafted subcutaneously is considerably higher than that of the tumours grafted intraperitoneally. This increased respiration is in the neighbourhood of 50 %.

A corresponding increase in the aerobic glycolysis amounts to about 30 %.

4. The anaerobic glycolysis is, on the average, of the same magnitude in each series.

Summary and discussion.

1. Estimations of the carbohydrate metabolism of several strains of mouse tumours are recorded. Great deviations from the standard values found for tumours of rat, fowl and a limited series of human tumours were observed in many cases.

Wide variations are shown to occur between tumours of different strains, and also between members of the same strain.

The most noticeable feature is the number of cases of high respiration, both in its absolute value and also in its relation to the aerobic and anaerobic glycolysis. This respiration is ineffective in checking the aerobic glycolysis, its activity in this direction being, in some cases, less than 10 % of that found in the case of working muscle, and in many mammalian tumours.

Some factors which might operate in causing these variations are changes in the respiratory quotient, differences of environment during growth, efficiency of blood supply, and the generally higher metabolic rate of the mouse as compared with larger animals.

2. A manometric method for the simultaneous measurement of the carbohydrate metabolism and the respiratory quotient is briefly described, based on the fact that the glycolysis effected by tumour tissue is a pure lactic fermentation.

The respiratory quotients with one exception were found to be below unity. This would tend to make the actual aerobic glycolysis relatively higher than that usually recorded, since the assumption has hitherto been made that a respiratory quotient of unity would result from the experimental conditions.

The results again illustrate the ineffectiveness of respiration in checking glycolysis.

3. Xylose is not metabolised by tumour tissue.

4. Evidence is brought forward which suggests that the glycolytic activity of tumours exerts a checking effect on their respiration.

5. The carbohydrate metabolism of tumours is to some extent influenced by the environment in which they grow. This is demonstrated by the study of two series of Jensen's rat sarcomata, simultaneously transplanted, one series subcutaneously and the other intraperitoneally.

The respiration of the subcutaneous growths was, on the average, 50 % higher than that of the intraperitoneal growths. The majority of these subcutaneous tumours do not exhibit a positive value for the excess fermentation, which was, until recently, regarded by Warburg as a criterion for the metabolism of tumour tissue.

The correlation of these differences with the normal tissue tensions of CO_2 and O_2 is difficult. Campbell found the oxygen tension in the abdominal cavity 50 % higher than under the skin, the CO_2 tensions being approximately the same.

The higher respiration found in these two series of tumours corresponds to the lower O_2 tension in the surrounding tissues, and *vice versa*. It is obvious that other factors which have not yet been analysed are operative.

The general result of these observations is to emphasise the difficulty of including the wide variations found in the carbohydrate metabolism of tumour tissue in one generalisation.

The constant factor is the possession of a high aerobic glycolysis, which, though not specific for tumour tissue, is a source of energy available for uncontrolled proliferation.

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